Carbohydrate Metabolism in Pregnancy

VI. PLASMA FUELS, INSULIN, LIVER COMPOSITION, GLUCONEOGENESIS, AND NITROGEN METABOLISM DURING LATE GESTATION IN THE FED AND FASTED RAT

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ABSTRACT The effects of late pregnancy on metabolic fuels, liver composition, gluconeogenesis, and nitrogen metabolism have been examined in fed and fasted rats.

Plasma free fatty acid (FFA) and immunoreactive insulin (IRI) are greater and glucose and ketones are lower in *fed* 19-day pregnant than they are in age-matched virgin rats. A 48 hr fast elicits greater increases in FFA and ketones and more profound reductions in glucose in the pregnant rats and obliterates the differences in IRI. Fetal weight is not modified by such fasting but maternal weight losses exceed that of the nongravid rats.

Livers from rats 19 days pregnant contain more and larger hepatocytes. Per μmole hepatic deoxyribonucleic acid (DNA)-phosphorus, water and protein are more abundant, whereas glycogen is unaffected. Livers from *fed* pregnant rats contain more lipid phosphorus and less neutral lipid fatty acid. After a 48 hr fast, hepatic steatosis supervenes in gravid animals due to accumulated neutral fat. The contents of hepatic acetyl-coenzyme A (CoA) and citric acid are not different in *fed* pregnant and virgin rats but are greater in the pregnant rats after fasting.

Formation of glucose-^{14}C and glycogen-^{14}C from administered pyruvate-^{14}C are the same in *fed* pregnant and virgin rats, but greater in the pregnant ones after a 24 or 48 hr fast.

Pregnancy does not affect creatinine excretion, and urinary urea is not different in *fed* pregnant, virgin, and postpartum animals. Contrariwise, more nitrogen, potassium, and phosphorus are excreted by the pregnant animals during a 2 day fast. The increment in urinary nitrogen is due largely to urea on the 1st day, whereas heightened ammonia accounts for half the increase on the 2nd and correlates with the enhanced ketonuria.

Muscle catabolism, gluconeogenesis, and diversion to fat are activated more rapidly and to a greater degree when food is withheld during late gestation in the rat. These catabolic propensities are restrained in the fed state. The capacity for "accelerated starvation" may confer survival benefit upon an intermittently eating mother in the presence of a continuously feeding fetus.

INTRODUCTION

Pregnancy poses unusual metabolic problems (1). Theoretically, the mother should be geared to conserve more exogenous nutrients whenever she eats in anticipation of the more severe demands which the presence of the conceptus confers upon her endogenous reserves whenever food is witheld. Thus, enhanced anabolism in the fed state should be associated with a heightened capacity for catabolism in the fasted state if fetal development and maternal survival are to be preserved under all conditions (1, 2). It has been known for some time that hyperphagia occurs during pregnancy and that net maternal weight gain is greater than can be ascribed to simple intrauterine growth (3–7). Increases in the size of certain structures, such as the liver (3, 5, 6, 8, 9) and net nitrogen retention (5, 10, 11), have also been

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described. On the other hand, catabolic processes have been characterized less fully, although enhanced mobilization of fat and ketonemia in the fasted pregnant animals have been recognized for many years (6, 12–14). The present studies were designed to examine these interrelationships in greater detail in the rat and to derive some inferences concerning the mechanisms by which they are mediated. Preliminary reports have been presented elsewhere (15, 16).

METHODS

Chemicals and reagents

Acetyl-coenzyme A (CoA) was purchased from Calbiochem, Los Angeles, Calif.; enzymes were obtained from Boehringer Mannheim Corp., New York; and other biologicals, such as pyridine nucleotides from Sigma Chemical Co., St. Louis, Mo. Reagents for glucose estimation (Glucostat) were secured from Worthington Biochemical Corp., Freehold, N. J. All organic chemicals were of reagent grade. Radioisotopes were purchased from New England Nuclear Corp., Boston, Mass. Silicic acid for lipid chromatography (minus 325 mesh) was obtained from Bio-Rad Laboratories, Richmond, Calif. and Hyflo Super-Cel (Celite Diatomite Filter Aid) from Johns-Manville Corp., New York. Duolite A-4 (anion exchanger) was obtained from Diamond Alkali Company, Calif., and Dowex 1×2-400 was purchased from Sigma Chemical Co.

Animals

Primiparous pregnant and age-matched virgin female rats were secured from Charles River Laboratories, Boston, Mass. Animals had been mated at 41–57 days of age. For individual experiments, the ages of pregnant and control animals matched within ±12 hr. Upon receipt in the laboratory, animals were maintained on Purina pellets and housed in individual cages as described elsewhere (17). Fasting animals were given unrestricted access to drinking water. All experimental manipulations (i.e. sacrifice, completion of urine collections, blood sampling, isotope injections, etc.) were performed between 9 and 11 a.m.

Plasma components

Blood samples were collected from the abdominal aorta into heparinized syringes and centrifuged immediately at 4°C. Animals had been anesthetized with Nembutal (40 mg/kg).

Plasma was deproteinized with $Ba(OH)_2$-$ZnSO_4$ and supernatants were analyzed for glucose (18) and total ketones (19). To estimate free fatty acids (FFA), plasma was extracted as per Dole (20). Heptane extracts were reduced to dryness under N_2 and resuspended in chloroform. Activated silicic acid (40–50 mg) and Hyflo Super-Cel (40 mg) were added to the chloroform to adsorb phospholipids. After mixing for 1 min, the tubes were centrifuged and aliquots of supernatant were analyzed for FFA by the Duncombe procedure (21). Recovery experiments indicated that 95.5–98.2% of the phospholipids were removed in this fashion and that 99.3–100.9% of added palmitic acid-^{14}C was recovered. Plasma urea and creatinine were estimated with the Technicon AutoAnalyzer by modifications of the procedures of Marsh, Fingerhut, and Kirsh (22) and Folin and Wu (23), respectively.

Immunoreactive insulin (IRI) was measured in the plasma by a double antibody technique (24) using human insulin as standard.

Analysis of liver

Animals were stunned by a blow on the head without prior anesthesia and aliquots of liver were analyzed as follows:

Nuclei. Nuclei were counted as per Weber and Cantero (25). Homogenates of liver (10% w/v in chilled 0.85% NaCl) were prepared with Teflon pestles in homogenizers immersed in ice. Homogenates were diluted 40-fold with freshly prepared staining solution (0.75 g orcein in 200 ml 45% glacial acetic acid (w/v) and filtered twice through Whatman 1 filter paper). Nuclei were counted in hemacytometer chambers and four chambers were analyzed to derive average values.

Composition. Livers were excised and frozen in liquid N_2 in less than 20 sec. Aliquots were analyzed for water and protein as detailed previously (17). Glycogen was precipitated from alkaline digests (26), resolubilized in water, and reprecipitated twice with ethanol for purification before acid hydrolysis (2.5 M H_2SO_4 for 2 hr at 100°C) and enzymatic assay with glucose oxidase. Total lipids were extracted with chloroform-methanol (2:1, v/v) and estimated gravimetrically after two washes as per Folch, Lees, and Stanley (27). Deoxyribonucleic acid phosphorus (DNA-P) was isolated from the residual pellet after lipid extraction by the method of Schmidt and Thannhauser (28); inorganic phosphorus was estimated (29) after digestion with 72% $HClO_4$.

Content of regulatory metabolites. Segments of liver were frozen *in situ* with aluminum clamps which had been chilled with liquid N_2 (30). Appropriate aliquots were analyzed for acetyl-CoA, citric acid, lipid phosphorus, and for esterified fatty acids in the neutral lipid fraction (i.e. equivalent principally to triglyceride fatty acids) as described elsewhere (17, 31).

Gluconeogenesis from labeled pyruvate in vivo

Unanesthetized animals were injected via tail vein with 0.5 ml of a solution containing 1 mmole sodium pyruvate and 5 μc pyruvate-3-^{14}C. 5, 10, 20, and 30 min thereafter, blood specimens (about 300 μl) were collected dropwise from the cut tip of the tail into the recesses of porcelain indicator plates which contained dried heparin. Protein-free filtrates (1:10) were prepared from each collection. After the 30 min specimen had been secured, animals were stunned by a blow on the head and sections of liver were frozen *in situ* with aluminum clamps (30) for subsequent isolation of labeled glycogen.

Analyses for glucose-^{14}C in the circulation. 1 ml portions of filtrate were passed over microcolumns (i.e. 1 ml tuberculin syringes filled to the 0.3 ml mark with Dowex 1×2-400, from the 0.3 to the 0.7 ml mark with activated Duolite A-4 [OH], and to the 1.0 ml mark with more Dowex 1×2-400 to remove labeled anionic and cationic components (32). Columns were rinsed with an additional 3 ml deionized distilled water, and eluates were counted and analyzed for glucose with glucose oxidase. Recovery of glucose-^{14}C added to blood before precipitation was 99.7–101.9% by this technique, whereas the recoveries of added alanine-^{14}C and pyruvate-^{14}C were less than 0.07% and 0.03%, respectively.

Since uncharged compounds other than glucose (such as glycerol) would also pass through the columns, their potential contribution was assessed by incubating eluates with sufficient hexokinase, glucose 6-phosphate dehydrogenase, and cofactors to convert all the glucose to 6-phosphogluconolac-

tone (and 6-phosphogluconic acid). After spectrophotometric documentation that the reaction had gone to completion, the mixtures were again passed through fresh columns. Less than 0.1% of the radioactivity present in initial eluates was recovered in the second eluates, thereby documenting that glucose-^{14}C is the only uncharged labeled compound that enters the circulation in meaningful amounts during the first 30 min after intravenous pyruvate-3-^{14}C.

Analyses for glucose-^{14}C in liver glycogen. Acid hydrolysates of glycogen precipitates were counted and analyzed for glucose with glucose oxidase. To document that all the radioactivity represented glucose-^{14}C, aliquots were neutralized with $Ba(OH)_2$, concentrated *in vacuo*, and chromatographed on paper in pyridine: isopropanol: glacial acetic acid: water (8:8:1:4) as described elsewhere (33). Glucose was localized by radioautography and eluted to determine specific radioactivity. Values agreed within ±3% with those obtained in the initial acid hydrolysates.

Radioactive assay. Solution for liquid scintillation counting was prepared by dissolving 15 g 2,5-diphenyloxazole (PPO), 150 mg *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP), and 240 g naphthalene in 3000 ml of xylene: dioxane: 95% ethanol (5:5:3; v/v). Up to 1 ml of aqueous sample could be solubilized in this mixture without appreciable compromise of counting efficiency.

Expression of results. The percentage of administered radioactivity converted to labeled glucose at various intervals after the injection of pyruvate-3-^{14}C was calculated on the basis of an assumed "glucose space" of 30% of body weight:

$$\text{per cent to glucose} = 100 \times \frac{\text{glucose space (ml)} \times \text{glucose-}^{14}\text{C in blood (cpm/ml)}}{\text{administered radioactivity (cpm)}}$$

The validity of this approach has been established by others (34); pregnancy does not appear to alter the percentage of body weight that is occupied by the glucose space (35, 36).

Incorporation of labeled pyruvate into hepatic glycogen was calculated as:

$$\text{per cent to glycogen} = 100 \times \frac{\text{liver weight (g)} \times \text{glucose-}^{14}\text{C in liver (cpm/g)}}{\text{administered radioactivity (cpm)}}$$

Specific radioactivity for glucose in the glucose space and liver glycogen has been expressed as counts per minute per microgram.

Nitrogen and mineral metabolism during fasting

Animals were housed in individual metabolism cages (Acme Metal Products, Inc., Chicago, Ill.) for the continuous collection of urines for 24-hr periods. Specimens were collected into receptacles containing 0.5 ml 6 N HCl and processed as described elsewhere (37). To maximize recovery of ammonia (38), urines in later experiments were secured under light mineral oil. Urinary total nitrogen (39), urea (22), creatinine (23), ammonia (40), and phosphorus (29) were determined by the Technicon AutoAnalyzer and sodium and potassium by flame photometry. Ketones were estimated by the method of Bessman and Anderson (19). Possible losses of ketones into mineral oil were evaluated by preparing 0.001 M solutions of acetone, β-hydroxybutyric acid and ethyl acetoacetate in 0.03 N HCl and storing these under mineral oil for 24 hr at room temperature. Recovery of the individual ketones in the aqueous phase after such mock collections exceeded 93%.

RESULTS

Gross changes in the mother during fasting in late pregnancy

Weight. 22 17-day pregnant rats, closely matched in age (65–72 days) and weight (289.7 ±6.3 g), were split into two groups: 11 were given continual access to feed, and 11 were fasted. Weights 48 hr later, on day 19 of pregnancy, are summarized in Table I. Fed animals gained an average of 28.1 g, whereas fasted animals lost an average of 35.4 g. Hysterectomy disclosed that fasting did not affect the average weights of the total conceptus although small but significant ($P < 0.02$) weight reductions were demonstrable in the nonfetal portion of the conceptus (i.e. Δ, Table I). In confirmation of others (4, 6), fetal mass was not diminished by maternal dietary deprivation during late gestation. Fed animals contained an average of 10.8 viable fetuses weighing 2.18 ±0.11 g each, whereas fasted animals average 10.6 viable fetuses weighing 2.16 ±0.09 g (Table I). Since average fetal weight on day 17 of gestation approximates 0.75 g (41), the net increment in total fetal mass per pregnant rat during the 48 hr in-

TABLE I

*Effect of 48 hr Fast in the Pregnant Rat (Day 17–19 of Gestation) on Weight of Mother, Conceptus, and Fetus**

Dietary status	Intact mother weight	Conceptus‡			Average fetus	
		Total	Fetuses	Δ	*n*	Weight
	g	g	g	g		g
Fed	316.9 ±4.4	44.2 ±1.2	23.5 ±4.5	20.7 ±0.8	10.8 ±1.6	2.18 ±0.11
Fasted	254.3 ±8.4	40.2 ±2.4	22.7 ±1.5	17.5 ±1.0	10.6 ±0.6	2.16 ±0.09
P	<0.001	NS	NS	<0.02	NS	NS

* Average values ±SEM observed on day 19 of gestation in pregnant rats which had been fasted for 48 hr ($n = 11$) or given continuing access to food ($n = 11$). *P* denotes significance of differences between fed and fasted groups; NS = not significant.
‡ The total conceptus was excised from each animal and weighed. Fetuses were then removed and weighed together. Δ denotes weight of the residual conceptus (i.e. uterus, placenta, and amniotic fluid) and was derived by difference.

TABLE II

*Effect of Pregnancy on Circulating Fuels and Insulin in Fed and 48-hr Fasted Rats**

Dietary status	Fed			Fasted		
Day of gestation	0	19	*P*	0	19	*P*
Plasma						
Glucose, *mg/100 ml*	108.6 ±4.6 (10)	80.9 ±2.8 (10)	<0.001	96.5 ±1.0 (11)	51.3 ±3.2 (8)	<0.001
FFA, *μEq/liter*	310 ±15 (7)	516 ±75 (7)	<0.02	443 ±21 (7)	739 ±59 (7)	<0.001
Ketones, *μEq/liter*	285 ±37 (7)	145 ±11 (7)	<0.01	1781 ±157 (7)	6473 ±549 (7)	<0.001
IRI, *μU/ml*	20.1 ±2.5 (10)	34.2 ±4.4 (10)	<0.02	9.7 ±1.1 (11)	12.8 ±2.1 (8)	NS
IRI/glucose, *μU/mg*	18.3 ±2.1	41.8 ±4.5	<0.001	9.9 ±0.9	23.9 ±3.5	<0.001

Abbreviations used: FFA, free fatty acid; IRI, immunoreactive insulin.

* Plasma specimens were secured on day 19 of gestation from pregnant rats which had been fasted for the preceding 48 hr (i.e. days 17–19 of gestation) or fed to the time of sacrifice. Age-matched virgin animals ("0 gestation") were processed concurrently in the same fashion as described in the text. Blood samples were collected from the aorta into heparinized syringes; animals were anesthetized with Nembutal (40 mg/kg body weight). *P* denotes significance of differences between mean ±SEM values for virgin and pregnant animals.

terval presumably averaged about 15 g. Thus, in fasted animals, the true loss of maternal mass must have been at least 50 g (i.e., 35.4 g plus 15 g). By contrast, when 11 age-matched virgin rats were subjected to a comparable 48 hr fast, weight loss averaged only 29.9 ±1.4 g (initial weight 189.4 ±1.6 g; final weight 159.5 ±2.2 g).

Circulating fuels and insulin. Separate experiments were performed with fed and 48-hr fasted, 19-day pregnant, and age-matched virgin rats (Table II). Plasma glucose ($P < 0.001$) and total ketones ($P < 0.01$) were lower and FFA higher ($P < 0.02$) in *fed* pregnant than virgin animals. After fasting, plasma glucose was lower and ketones and FFA were higher in both groups. However, the excursions were more pronounced in the pregnant animals: Their blood sugar declined to near hypoglycemic levels ($P < 0.001$); their FFA rose twice as much ($P < 0.001$); and their increments in plasma ketones were 4-fold greater ($P < 0.001$) (Table II). The findings are in agreement with earlier observations by Scow, Chernick, and Brinley (6).

In contrast to the findings of others (42), concentrations of plasma IRI were substantially greater in fed pregnant than in fed virgin animals ($P < 0.02$) (Table II). After a 48 hr fast, levels of plasma IRI in the two groups no longer differed significantly (Table II). However, by virtue of the profound fall in glucose, the fasted pregnant rats continued to display elevated ratios of plasma IRI: glucose ($P < 0.001$) (Table II). These IRI measurements do not support the postulate (6) that the marked decline of blood sugar causes absolute hypoinsulinism in fasted pregnant rats.

Conclusions. Fed rats in late gestation exhibit increased levels of plasma IRI in association with lowered plasma glucose and ketones. Plasma FFA are slightly elevated as if mobilization of fat were being driven by factors incompletely restrained by the "extra" insulin. When all food is withheld from such *previously well-fed* mothers, fetal growth is not modified. However, maternal weight loss exceeds that of comparably fasted nonpregnant animals presumably because endogenous reserves are taxed more severely. Hyperlipacidemia and ketonemia are also more pronounced, and plasma glucose falls precipitously. Absolute differences in IRI are obliterated by fasting so that peripheral tissues of fasted pregnant animals are no longer exposed to relatively supranormal concentrations of insulin.

Effects of pregnancy and fasting on the liver

In view of the role of the liver in gluconeogenesis and in lipid metabolism, livers from 19-day pregnant and age-matched virgin rats were analyzed after 48 hr fast or uninterrupted access to feed. Previous reports have indicated that rat livers are larger during gestation (3, 5, 6, 8, 9) and that hepatic DNA and ribonucleic acid (RNA) are increased (9, 43). However, cell numbers have not been quantified, and detailed characterizations of the effects of starvation have not been provided previously.

Liver cellularity. Livers from pregnant animals weighed more under all conditions ($P < 0.001$) (Table III) and contained more DNA-P ($P < 0.01$ in fed, $P < 0.05$ in fasted animals) and nuclei ($P < 0.05$ in fed animals) (Table III). Since the ratio of DNA-P: nucleus was not altered by pregnancy (Table III), these increases are consistent with cellular hyperplasia. Some cellular hypertrophy may also be inferred since the DNA-P per gram and the nuclei per gram of liver (wet weight) were consistently smaller in pregnant animals (Table III).

TABLE III

*Effect of Pregnancy and Fasting on the Liver**

Dietary status	Fed			Fasted		
Day of gestation	0	19	*P*	0	19	*P*
Body weight, *g*	211.8 ±5.8 (12)	309.1 ±8.9 (12)	<0.001	174.1 ±4.9 (12)	249.1 ±7.8 (12)	<0.001
Liver weight, *g*	8.78 ±0.40 (12)	12.98 ±0.50 (12)	<0.001	5.60 ±0.10 (12)	8.90 ±0.28 (12)	<0.001
Liver cellularity‡						
DNA-P, *μmoles/g*	6.99 ±0.21 (12)	5.92 ±0.10 (12)	<0.001	10.16 ±0.15 (12)	8.11 ±0.20 (12)	<0.001
Total DNA-P, *μmoles/liver*	56.1 ±1.9 (12)	74.2 ±1.8 (12)	<0.001	55.7 ±1.3 (12)	68.8 ±2.1 (12)	<0.001
Nuclei, 1×10^8/*g*	2.31 ±0.14 (6)	1.93 ±0.06 (6)	<0.001	3.45 ±0.26 (6)	2.62 ±0.09 (12)	<0.05
Total Nuclei, 1×10^8/*liver*	20.4 ±1.4 (6)	24.7 ±1.0 (6)	<0.05	19.9 ±1.5 (6)	22.9 ±0.8 (6)	NS
Liver composition (*concentration/μmole DNA-P*)						
Water, *mg*	94.4 ±0.6 (6)	114.8 ±3.0 (6)	<0.001	67.7 ±1.0 (6)	81.4 ±0.9 (6)	<0.001
Protein, *mg*	19.8 ±0.3 (6)	24.6 ±0.9 (6)	<0.001	17.5 ±0.3 (6)	20.1 ±0.5 (6)	<0.001
Glycogen, *mg*	5.61 ±0.52 (6)	5.11 ±0.46 (6)	NS	0.27 ±0.006 (6)	0.41 ±0.11 (6)	NS
NL esterified FA, *μmole*§	5.21 ±0.56 (6)	3.16 ±0.53 (6)	<0.01	3.04 ±0.43 (6)	10.57 ±1.05 (6)	<0.001
Phospholipid-P, *μmoles*	5.70 ±0.16 (6)	7.01 ±0.03 (6)	<0.001	4.96 ±0.16 (6)	6.76 ±0.09 (6)	<0.001
Acetyl-CoA, *mμmoles*	7.81 ±1.70 (6)	7.72 ±0.56 (6)	NS	5.95 ±0.43 (6)	7.56 ±0.37 (6)	<0.02
Citric Acid, *mμmoles*	61.7 ±3.7 (6)	52.4 ±2.5 (6)	NS	13.6 ±0.3 (6)	17.7 ±0.6 (6)	<0.001

* Animals were sacrificed without anesthesia by cervical fracture. Conditions of fasting and feeding for pregnant and age-matched virgin control animals are as described in Table II. *P* denotes significance of difference between mean ±SEM values for virgin and pregnant animals.

‡ Values derived on the basis of initial wet weight of liver.

§ NL esterified FA denotes fatty acids derived by saponification of the neutral lipid fraction.

During fasting, liver weight was reduced in pregnant ($P < 0.001$) as well as virgin ($P < 0.001$) animals (Table III). The losses presumably reflected diminution of cytoplasm within individual hepatocytes, since the number of nuclei [1] per liver and micromoles DNA-P per liver were not diminished significantly by fasting in either group (Table III).

Liver composition and regulatory metabolites. Results are summarized in Table III. In view of the effects of pregnancy and starvation on cell size, all values have been expressed as concentration per micromole DNA-P. It was felt that this provided a better index of the abundance per hepatocyte than more conventional reference standards such as wet or dry weight.

Liver composition. Hepatic water and protein were increased by pregnancy in fed ($P < 0.001$) as well as fasted ($P < 0.001$) rats, whereas glycogen was not affected significantly (Table III). Gravimetrically determined total fat did not differ significantly in fed pregnant vs. virgin animals (7.44 ±0.4 vs. 6.48 ±0.28 mg/μmoles DNA-P, respectively). However, lipid fractionation disclosed that this seeming equivalence resulted from a diminution of neutral lipid (NL)–esterified fatty acids ($P < 0.01$) in association with an increase in phospholipid P ($P < 0.001$) in the livers of the pregnant animals (Table III). After 48 hr fast, total fat was significantly greater in pregnant than in virgin livers (8.34 ±0.3 vs. 5.33 ±0.21 mg/μmoles DNA-P, respectively, $P < 0.001$). The relative steatosis resulted from a profound accumulation of NL esterified fatty acids ($P < 0.001$) and a persistent increase in phospholipid P ($P < 0.001$) (Table III).

Since the hepatic content of acetyl-CoA and citric acid can exert "regulatory" effects upon key enzyme activities (44, 45) (e.g. pyruvate carboxylase and pyruvate dehydrogenase in the case of acetyl-CoA; acetyl-CoA carboxylase and phosphofructokinase in the case of citric acid), "freeze-clamped" portions of liver were analyzed for these metabolites. Acetyl-CoA and citric acid did not differ in *fed* pregnant and virgin animals (Table III) despite the increased plasma FFA in the former (Table II). On the other hand, *after 48 hr fast,* livers from pregnant animals contained more acetyl-CoA ($P < 0.02$) and citric acid ($P < 0.001$) than those from fasted nongravid rats (Table III). However, within the pregnant group, the content of acetyl-CoA (millimicromoles/μmole DNA-P) was not different in the fasted than in the fed state, and the content of citric acid fell (Table III).

Conclusions. Rat livers in late gestation are heavier and contain more and larger hepatocytes. Theoretically, these anatomical features might confer greater biosynthetic efficiency if hepatic perfusion per unit mass is not diminished in vivo. Water and protein in the liver of the pregnant rat are increased in the fed as well as the

[1] Histological examinations for diploid nuclei were also performed on 4-mμ sections of liver stained with hematoxylin and eosin. Independent counts by two observers failed to disclose differences between livers from pregnant and virgin animals. The number of diploid nuclei ranged from 4 to 10% in both groups. Thus, counts of nuclei and estimates of DNA-P seem to provide valid indices of cellularity under these conditions.

TABLE IV

*Effects of Pregnancy on the Appearance of Glucose-^{14}C in the Circulation and Hepatic Glycogen after the Intravenous Administration of Pyruvate-3-^{14}C**

	Day of gestation	n		Circulating glucose, min after pyruvate-3-^{14}C 5	10	20	30	Glycogen, 30 min after pyruvate-3-^{14}C
(A) Fed animals								
Formation of glucose-^{14}C, % *administered counts*								
	0	8		2.49 ±0.23	3.02 ±0.27	3.67 ±0.51	3.46 ±0.37	0.061 ±0.012
	19	6		2.46 ±0.31	3.51 ±0.43	3.75 ±0.41	3.68 ±0.39	0.090 ±0.015
			P	NS	NS	NS	NS	NS
Glucose SA, *cpm/mg*								
	0	8		3,282 ±226	4,070 ±267	4,912 ±417	4,655 ±440	21.5 ±3.6
	19	6		3,080 ±268	4,091 ±326	4,531 ±314	4,374 ±341	27.4 ±3.0
			P	NS	NS	NS	NS	NS
(B) After 24 hr fast								
Formation of glucose-^{14}C, % *administered counts*								
	0	6		3.33 ±0.35	5.66 ±0.34	8.54 ±0.92	9.60 ±0.91	0.241 ±0.080
	19	6		7.29 ±0.75	9.55 ±0.82	12.50 ±1.41	12.11 ±1.51	3.392 ±0.551
			P	<0.001	<0.01	<0.05	NS	<0.001
Glucose SA, *cpm/mg*								
	0	6		6,538 ±853	11,570 ±879	14,950 ±925	16,140 ±1,211	4,101 ±137
	19	6		12,930 ±852	16,030 ±950	19,100 ±1,977	17,880 ±1,701	4,940 ±1,540
			P	<0.001	<0.01	NS	NS	<0.01
(C) After 48 hr fast								
Formation of glucose-^{14}C, % *administered counts*								
	0	8		3.08 ±0.46	5.15 ±0.46	8.26 ±0.69	8.58 ±0.61	0.501 ±0.102
	19	6		5.70 ±0.58	8.15 ±0.30	10.27 ±0.70	10.13 ±0.50	6.021 ±1.230
			P	<0.01	<0.001	NS	NS	<0.001
	21	8		5.88 ±0.68	8.73 ±0.70	10.86 ±1.05	10.51 ±0.76	1.871 ±0.425
			P	<0.01	<0.001	NS	NS	<0.01
Glucose SA, *cpm/mg*								
	0	8		6,419 ±917	10,540 ±990	15,410 ±1,093	16,840 ±1,024	2,976 ±595
	19	6		12,890 ±1,036	17,740 ±515	20,780 ±738	19,570 ±636	16,320 ±3,335
			P	<0.001	<0.001	<0.01	<0.05	<0.001
	21	8		15,040 ±1,554	19,550 ±1,316	21,330 ±1,401	21,780 ±1,076	10,160 ±2,283
			P	<0.001	<0.001	<0.01	<0.001	<0.01

* Pregnant rats (on the 19th day of gestation) and age-matched virgin control animals were injected intravenously with 1 mmole pyruvate-3-^{14}C. Animals had been allowed free access to food ("fed") or fasted for the preceding 24 or 48 hr. Serial blood specimens were obtained from the cut tip of the tail 5, 10, 20, and 30 min after the administration of pyruvate-3-^{14}C. Livers were secured for glycogen analysis immediately after the last collection of blood. For the experiments with 48-hr fasts, additional pregnant animals were also evaluated on day 21 of gestation after food had been withheld from days 19 to 21. Values for the formation of glucose-^{14}C have been derived as discussed in text. Specific activities have been adjusted to an initial value of 1.0×10^7 cpm/mmole for the injected pyruvate-3-^{14}C. Results have been expressed as mean ±SEM. n = number of animals in each group; P denotes statistical significance of the difference between pregnant and virgin animals.

fasted state. However, liver fat displays certain dietary dependencies; "storage" lipids (i.e. NL esterified fatty acids) are diminished and "membrane" lipids (i.e. phospholipids) are increased in *fed* pregnant animals, whereas the former type of lipids accumulate during fasting. Despite this steatosis, the hepatic content of acetyl-CoA is not augmented and citric acid is diminished. Thus, unless compartmentation is present, allosteric inhibition of phosphofructokinase by citric acid or activation of pyruvate carboxylase by acetyl-CoA cannot be sustaining the changes in hepatic glycolysis or gluconeogenesis that may have been established after the 48 hr fast in late pregnancy.

Gluconeogenic efficiency in vivo

Virgin and pregnant rats were injected with 1 μmole pyruvate-3-^{14}C, intravenously. Such large doses simplify the evaluation of net gluconeogenic potential since they

"load" gluconeogenic mechanisms and offset variations in the availability of endogenous precursors (34). Intravenous rather than intraperitoneal (34) administration was selected to eliminate the problems in absorption that might be posed by gravidity. Sampling was performed sufficiently soon after injection (i.e. 5–30 min) to minimize artifacts due to reutilization of labeled glucose. Results are summarized in Table IV.

Fed animals. Within the first 30 min after the administration of pyruvate-3-^{14}C, less than 5% of the radioactivity was converted into labeled glucose (Table IV A). Absolute rates of incorporation and specific radioactivities for circulating glucose and hepatic glycogen in fed 19-day pregnant and age-matched virgin rats did not differ significantly (Table IV A).

Fasted animals. After a 24 hr (Table IV B) or 48 hr (Table IV C) fast, the incorporation of radioactivity into glucose-^{14}C was increased. However, more radioactivity was incorporated in the pregnant than in the nongravid animals during the first 5 ($P < 0.001$), 10 ($P < 0.01$), and 20 ($P < 0.05$) min following pyruvate administration after 24 hr fast (Table IV B), and during the first 5 ($P < 0.01$) and 10 ($P < 0.001$) min following pyruvate after 48 hr fast (Table IV C). Since differences in rates of glucose utilization would least affect these early time points, the findings are compatible with a greater proportion of the administered pyruvate being converted to glucose at a more rapid rate in the fasted pregnant animals.[9] Similar conclusions may be derived on the basis of specific radioactivity; circulating glucose contained more counts per minute per milligram in pregnant than in virgin animals 5 ($P < 0.001$) and 10 ($P < 0.01$) min after pyruvate administration after 24 hr fast (Table IV B) and in all specimens after 48 hr fast (Table IV C).

The differences between gravid and nongravid fasted animals could not be ascribed to sequestration of glucose-^{14}C as glycogen in livers of the latter. Formation of glycogen-^{14}C was 14-fold greater in pregnant than nongravid animals after 24 hr fast (Table IV B), and 12-fold and 4-fold greater in the 19 and 21-day pregnant rats, respectively, after 48 hr fast (Table IV C). The specific radioactivities for hepatic glycogen were higher in all the fasted pregnant groups (Table IV-B and C). The mechanism underlying the glycogenesis after pyruvate administration are unclear. Preliminary evidence suggests that the concurrent rises in plasma insulin may be implicated.

Conclusions. Gluconeogenesis per hepatocyte may be restrained in the *fed* pregnant rat. Despite their larger livers, such animals do not form glucose-^{14}C or glycogen-^{14}C more rapidly than age-matched virgin animals, a finding that is consistent with the earlier in vitro observation that less pyruvate is converted to glucose per milligram wet weight of liver slices from fed pregnant rats (49). On the other hand, *fasted* pregnant animals display more gluconeogenic potential from three carbon precursors than nongravid controls. They convert a greater fraction of pyruvate "loads" to circulating glucose at a more rapid rate. Whether the heightened efficiency is due to enhanced function per individual hepatocyte, increases in total liver mass, or heightened contributions from renal gluconeogenesis cannot be determined from the available data. However, the acute and "loading" nature of the challenge renders it unlikely that differences in the extrahepatic oxidative disposition of pyruvate can be assigned a major role. Fasted pregnant rats also incorporate more of the newly formed glucose into hepatic glycogen. Thus, livers from fasted pregnant animals may be poised for more effective glycogenic repletion when exogenous nutrients again become available.

Nitrogen and electrolyte metabolism during fasting

Although the above experiences with exogenous pyruvate indicate *enhanced gluconeogenic potential* during fasting in late pregnancy, they need not be equated with an absolute increase in gluconeogenesis. Net formation of glucose from *endogenous* resources may be rate-limited by the generation of appropriate gluconeogenic amino acids in the periphery, their availability to gluconeogenic sites, and their preliminary processing (i.e. deamination or transamination) within such sites. Several of these steps may be attenuated during late pregnancy. Plasma amino acids are diminished in the gravid rat (5), as in the pregnant primate (50), and hepatic alanine α-ketoglutarate transaminase activity is reduced (5). To assess the integrated consequences of all these endogenous changes, 24-hr urinary excretion patterns were monitored during 2 days of fasting. Optimum sensitivity was sought by studying the same animals antepartum (AP) from days 19 to 21 of gestation, as well as postpartum (PP) from days 10 to 12 after delivery, thus enabling each animal to serve as her own control.

Urinary nitrogen. Essentially similar results were obtained in several experiments. However, since recoveries for ammonia were more complete when urines were collected under oil, only these studies are presented

[9] Studies based on direct assay for relevant enzymes in liver suggest that meaningful gluconeogenesis does not occur in the rat fetus (46, 47). Recent infusion studies in the intact animal have challenged this thesis (36) and limited gluconeogenesis has been demonstrated with fetal rat kidney cortex in vitro (48). The present data do not resolve the controversy. However, since the 21 day fetus is more than twice as big as the 19 day, and more mature enzymatically (46, 47), our finding of essentially similar values for glucose-^{14}C *at all time points* in the 19 and 21 day pregnant rat (Table IV C) would indicate that the glucose-^{14}C must originate predominantly via gluconeogenesis by the mother.

TABLE V

*The Effect of Pregnancy on Urinary Excretion of Nitrogen, Electrolytes, and Ketones During Fasting**

Dietary status	24-hr urinary excretions														
	Fast, day 1							Fast, day 2							
Day of gestation	0			20			PP 11	0			21			PP 12	
Nitrogen															
n	5		P	8		P	8	5		P	8		P	8	
Total N, *mg*	220	±14	++	286	±14	++	210 ±19	180	±6	++	233	±16	++	172	±8
Urea N, *mg*	174	±15	+	221	±12	+	166 ±14	139	±7	NS	143	±11	NS	125	±7
Anmonia N, *mg*	12.9	±0.8	++	17.8	±1.3	+++	9.1 ±1.0	17.9	±1.4	+++	44.2	±2.1	+++	15.3	±1.4
Creatinine N, *mg*	3.42	±0.22	NS	3.14	±0.07	NS	2.92 ±0.16	3.43	±0.13	NS	3.18	±0.12	NS	3.11	±1.03
Electrolytes															
n				7			7				7			7	
Sodium, *mEq*				5.88	±0.94	NS	8.10 ±1.70				4.08	±0.68	NS	3.54	±1.22
Potassium, *mEq*				15.3	±1.1	+	11.4 ±1.4				10.3	±1.6	+	4.2	±0.8
Phosphorus, *mg*				28.8	±1.1	++	18.6 ±2.2				21.5	±0.9	++	14.8	±1.4
Ketones															
n	5			7			7	5			7			7	
Total, *μmoles*	22	±7	+++	595	±139	+++	26 ±2	47	±6	+++	3211	±606	+++	59	±9

* The table compares 24-hr urinary excretions (mean ±SEM) in the same animals during 2 days of fasting while pregnant (i.e. from day 19 to 20 and 20 to 21 of gestation) with postpartum (i.e. PP; from day 10 to 11 and 11 to 12 after delivery). Values are also listed for concurrently fasted age-matched virgin rats (i.e. 0 gestation). *n* denotes the number of animals in each group. Statistical significance (*P*) of the differences between virgin vs. pregnant, and pregnant vs. postpartum (*PP*) are denoted by the following plus signs between appropriate columns: + = < 0.05, ++ = < 0.01, and +++ = < 0.001; NS = not significant.

in Table V. Total urinary nitrogen excretion was greater AP than PP ($P < 0.01$) on each of the 2 days of fasting. The differences cannot be ascribed to postpartum nitrogen retention since values for PP animals were not significantly different than those observed in a smaller group of fasted, age-matched, virgin animals (Table V).

In all groups, more than 80% of total urinary nitrogen could be accounted for as urea, ammonia, and creatinine (Table V). Creatinine excretion did not differ in virgin, AP, and PP animals. Contrariwise, urea and ammonia displayed striking differences; both were significantly greater during the 1st day of fasting in the AP animals, and on a quantitative basis, the heightened excretion of urea accounted for most of the increase in total urinary nitrogen (Table V). However, during the 2nd day, urinary urea diminished in all groups, and significant differences between AP vs. PP and virgin animals could no longer be demonstrated. Instead, far more sizeable differentials became manifest in urinary ammonia (Table V). AP animals excreted about 28 mg ammonia N more than PP or virgin animals ($P < 0.001$), a difference sufficient to account for approximately half of their increase in total nitrogen excretion at this time.

Similar gestational effects upon amino acid catabolism were not demonstrable in the fed state. Urines (24-hr) collected during unrestricted access to food from five virgin animals and eight gravid rats from days 18 to 19 AP and days 9 to 10 PP were analyzed for urea and creatinine. Urea N (mg/24 hr) averaged 360 ±14, 370 ±36, and 360 ±20, and creatinine N (mg/24 hr) averaged 4.67 ±0.14, 4.09 ±0.21, and 3.91 ±0.13 in the *fed* virgin, AP, and PP animals, respectively.

To preserve basal conditions, none of the above animals were bled. Instead, plasmas for the estimations of urea and creatinine were secured from age-matched gravid and nongravid groups under similar experimental conditions. Plasma urea and creatinine in the fed state, or after a 24 or 48 hr fast, were not significantly different in AP, PP, or virgin animals (Table VI). Average creatinine clearances were derived on the basis of these plasma values and the urinary excretions shown in Table V. Despite the complexities in interpretation, our values for endogenous creatinine clearances were similar in AP, PP, and virgin animals and concorded closely with previous estimates of rat glomerular filtration rates based on inulin in nongravid and 20-day pregnant rats (51). Thus, gestational changes in glomerular filtration do not constitute a likely explanation for the heightened excretion of nitrogen during fasting.[3]

Urinary electrolytes. Previous reports have indicated

[3] The failure of the rat kidney to increase in size during pregnancy (3, 6) concords with this impression. Moreover, unlike the liver (5), the kidney of the pregnant rat also does not exhibit any reduction in alanine α-ketoglutarate transaminase activity (5).

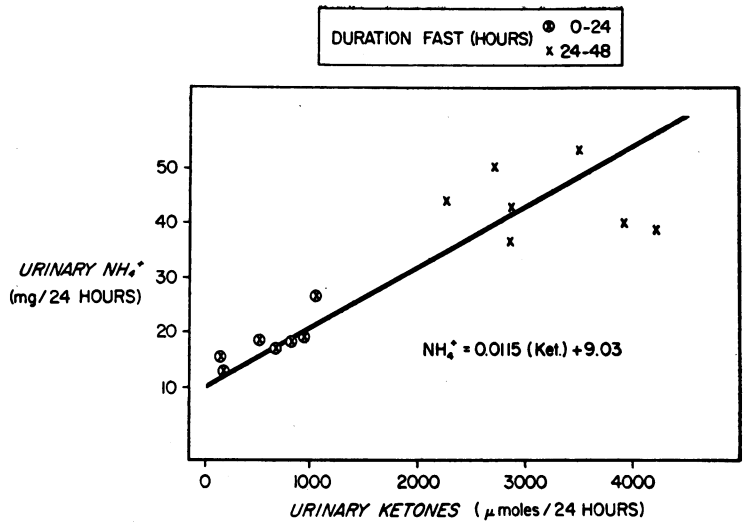

FIGURE 1 Pregnant rats were fasted for 48 hr from day 17 to 19 of gestation. Values for urinary ammonia and ketones during each 24 hr period are depicted above.

that urinary excretion of potassium is not modified in *fed* 20-day pregnant rats, although tubular reabsorption of sodium may be enhanced (51). In the present studies, with *fasted* animals, urinary sodium was not significantly different AP than it was PP, whereas AP animals excreted significantly more potassium ($P < 0.05$) and phosphorus ($P < 0.01$) on each of the 2 days of fasting (Table V).

TABLE VI

*Effect of Pregnancy on Plasma Creatinine and Urea Nitrogen in Fed and Fasted Rats**

Day of gestation‡	Days of fast	n	Plasma creatinine	P	Plasma urea N	P
			mg/100 ml		*mg/100 ml*	
0	0	5	0.39 ±0.02		16.7 ±2.1	
18	0	6	0.36 ±0.01	NS	18.7 ±1.2	NS
19	0	6	0.41 ±0.06	NS	18.5 ±0.5	NS
PP 10	0	6	0.39 ±0.02	NS	19.7 ±2.3	NS
0	1	6			15.1 ±0.9	
20	1	6	0.44 ±0.01		14.8 ±1.0	NS
PP 11	1	5	0.44 ±0.08		15.2 ±0.9	NS
0	2	5	0.40 ±0.02		14.0 ±1.6	
21	2	4	0.48 ±0.04	NS	16.6 ±1.8	NS
PP 12	2	8	0.47 ±0.03	NS	13.9 ±0.6	NS

* Animals were anesthetized with Nembutal (40 mg/kg) and plasma was secured as described in the text.

‡ Day of gestation: 0 denotes age-matched virgin animals; PP denotes days after delivery (i.e. postpartum). *P* compares statistical significance of values (mean ±SEM) in pregnant and PP with virgin animals; NS = not significant.

The ratios for urinary phosphorus: nitrogen in our fasted AP animals (Table V) approximated the relationships in rat muscle (52), and hence are compatible with augmented muscle catabolism. On the other hand, the ratios for potassium: nitrogen (Table V) exceeded the interrelationships in muscle (52) so that some of the kaliuresis must have originated from additional sources besides the breakdown of muscle mass.

Urinary ketones. In view of the exaggerated ketonemia (Table II), and heightened urinary excretion of ammonia (Table V) in *fasted* pregnant rats, and the recent evidence that acidosis may "trigger" a coupled activation of renal ammoniagenesis and gluconeogenesis (53, 54), total urinary ketones were quantified. As others have shown (6, 14), fasting gravid rats displayed dramatic increases in ketonuria; excretions were 30- and 60-fold more abundant in AP than PP or virgin animals during the 1st and 2nd day of fasting, respectively (Table VI) and correlated ($P < 0.001$) with urinary ammonia (Fig. 1).

Conclusions. As judged by the exaggerated urinary losses of nitrogen, potassium, and phosphorus, fasting in late pregnancy is attended by greater catabolism than under nongravid conditions, a finding consistent with our earlier postulation of "accelerated starvation" during gestation (1). The characteristic progression from hepatic to renal gluconeogenesis during starvation (55) does not appear to be modified qualitatively. Urinary urea declines and ammonia increases on the 2nd day of fasting in gravid as in virgin animals. However, quantitatively, the urinary excretion patterns are compatible with gestational increases in amino acid traffic at both

gluconeogenetic sites. These propensities seem to be restrained in fed animals. Despite heightened intake of food and increased liver mass, urinary urea in *fed* pregnant rats does not differ from that seen in nongravid animals.

DISCUSSION

We have demonstrated that no aspect of catabolism (with the possible exception of heightened plasma FFA) is increased in the fed pregnant rat, whereas all diversion to endogenous fuels is exaggerated when food is withheld. Although precise mediation remains to be clarified, certain speculations may not be inappropriate.

Pregnancy is attended by two novel features. Firstly, the growing fetus poses an ever-increasing drain upon maternal fuels, and the uninterrupted fetal development that occurs when the previously well-fed mother is fasted would suggest that these demands cannot be fulfilled wholly from maternal fat. Indeed, experiments comparing the effects of excising the fetus alone with removal of the fetus plus the placenta have indicated that the fetus is responsible for the lowering of plasma amino acids[4] (56) and plasma glucose (61) that are observed after an 18 hr fast in the 21-day pregnant rat. It is tempting to suggest that the hypoaminoacidemia could sustain a further self-perpetuating egress of amino acids from maternal muscle via the type of negative feed-back that has been postulated when certain circulating amino acids fall after the administration of progesterone (60) or during normal starvation (62).

Secondly, the placenta develops as a new endocrine structure. It elaborates hormones such as estrogens and progesterone directly or sustains their elaboration from the ovary via a peptide which can exhibit luteotropic as well as mammotropic and growth hormone–like properties (placental lactogen) (63). In most species, these secretions parallel the growth of the conceptus (64–68).[5] More importantly, their elaboration is unremitting and not subject to feed-back regulation by excursions in circulating metabolites (66, 67).

The peptide has displayed lipolytic properties in vitro (74) and a capacity to potentiate the actions of growth hormone (75); administration of the peptide or female sex steroids has diminished the responsiveness to the blood sugar–lowering actions of insulin and enhanced the pancreatic release of insulin (76–81). Moreover, at least in man, progesterone has exhibited distinctly catabolic effects upon protein metabolism (59, 60). Thus, although the precise quantitative contribution of each of these hormones to the metabolic changes of pregnancy has not been established, it seems likely that their continuous integrated action may be implicated in (*a*) the activated lipolysis in isolated adipose tissue from the *fed* pregnant rat (82); (*b*) the increase in the plasma FFA of such animals (*vide supra,* and reference 6); (*c*) their blunted hypoglycemic response to standard doses of exogenous insulin administered in vivo (16); and (*d*) the hyperplasia of their pancreatic islets (see reference 83 for review of literature).

The restraining effects of insulin upon lipolysis, hepatic ketogenesis, hepatic gluconeogenesis, and release of amino acids from muscle are well established (84). We have demonstrated that circulating IRI is supranormal in fed pregnant rats, as in postprandial gravid humans. Although we have not excluded the possibility that some of this "extra" IRI may represent less potent, immunologically reactive precursors of insulin, our analyses would indicate that the "IRI" exerts sufficient biological action to brake ketogenesis, gluconeogenesis, urea formation, kaliuresis, phosphaturesis, etc. The "insulinization," especially in the liver may even be implicated in the significant lowering of blood sugar in the *fed* pregnant rat. On the other hand, we have shown that IRI falls, perhaps as a consequence of the further lowering of blood sugar, when food is withheld so that absolute concentrations of IRI are no longer greater in pregnant than in nongravid animals. Under such circumstances, all the adaptations to starvation (i.e. mobilization of fat, activation of gluconeogenesis, ketogenesis, muscle breakdown, etc.) are triggered more rapidly and more severely in the pregnant animal. It is as if the "normal" levels of IRI are insufficient to restrain the continuous activity of opposing catabolic forces. Despite augmented efficiency, maternal gluconeogenesis appears unable to "keep up" with the continuing siphoning of glucose and the precursors for gluconeogenic repair by the conceptus. Thus, blood sugar may approach the hypoglycemic range, and additional facilitators of pe-

[4] The well-documented aminoaciduria of pregnancy (57, 58) and the proposed direct effects of progesterone on hepatic handling of amino acids (59, 60) could also contribute to gestational reductions in plasma amino acids. As yet, these phenomena have not been evaluated in the rat.

[5] The situation is somewhat complicated in the rat. Whereas primates display increasing plasma levels of placental lactogen throughout gestation (64–68), a luteotropic-mammotropic principle (69) is maximally present in the rat placenta and circulation only in midpregnancy (70), and plasmas from 17- to 19-day pregnant rats do not contain increased material which is immunologically cross-reactive with rat growth hormone (71). However, some luteotropic-mammotropic activity is still present in the placenta of the 17 day pregnant rat (72), and alkaline extracts from placentas of 17- to 18-day pregnant rats can increase liver RNA (43). Moreover, recent reports indicate that "antiserum prepared against human placental lactogen cross reacts with a rat placental extract obtained on the eighteenth day of gestation" (42). Presumably, it is this material, acting through the ovary (4, 43), which can maintain steroidogenesis and pregnancy in the rat after hypophysectomy in midgestation (69, 73).

ripheral catabolism and gluconeogenesis, such as catecholamines, may be called into play (38).

What physiological ends are subserved in this fashion? One may suggest that the potentiality for "accelerated starvation" is protective for mother as well as fetus. With an intermittently eating mother, and a continuously feeding fetus, a metabolic setting for rapid transfer to maternal fat would be highly desirable. It would afford maximal conservation of maternal glucose and gluconeogenic precursors when exogenous nutrients are withheld and assure their availability for the maternal brain and fetal tissue. The increasing placental elaboration of metabolically active principle with contrainsulin as well as insulinogenic potentialities in parallel with the growth of the fetus would provide just the right temporal juxtaposition to make it all work. In such a formulation, the "extra" insulin which is required for anabolism whenever the mother eats would represent the overhead that she must pay for survival in the fasted state.

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